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Intensification of enzymatic conversion of glucose to lactic acid by reactive extraction

Kailas L. Wasewar^a, Vishwas G. Pangarkar^{a,*}, A. Bert M. Heesink^b, Geert F. Versteeg^b

^a*Institute of Chemical Technology, Mumbai University, Matunga, Mumbai 400 019, India*

^b*Department of Chemical Engineering, University of Twente, 7500 AE, Enschede, The Netherlands*

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Abstract

Lactic acid is an important commercial product and extracting this out of aqueous solution is a growing requirement in fermentation-based industries and recovery from waste streams. The design of an amine extraction process requires (i) equilibrium and (ii) kinetic data for the acid–amine (solvent) system used. Equilibria and kinetics for lactic acid extraction by Alamine 336 in octanol as a diluent have been determined and compared with other diluents studied earlier. An approach for extracting the lactic acid by a long-chain tertiary amine, which is in the dispersed phase as a liquid ion exchanger (LIX), is presented. A mathematical model for slurry phase reactor with glucose in the continuous aqueous phase, the amine with a diluent in the dispersed phase and the immobilized enzyme as the solid catalyst, has been developed using equilibrium and kinetic data for reactive extraction. Effects of various parameters affecting the conversion of glucose have been discussed. The model has been solved for batch and semi-batch modes. It has been shown that the semi-batch mode yields approximately five times higher productivity than batch mode.

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Keywords: Lactic acid; Reactive extraction; Fermentation; Alamine 336; Octanol; Equilibria

1. Introduction

Lactic acid is a chemical utilized in the food, chemical and pharmaceutical fields. In particular, an increasingly interesting application is the use of lactic acid as a monomer for the synthesis of biodegradable homopolymers and copolymers (Buchta, 1983; Datta, Tsai, Bonsignore, Moon, & Frank, 1995). A growing demand for biodegradable polymers, both substitutes for conventional plastic materials and new materials for specific uses such as controlled drug delivery or artificial prostheses, draws attention to improving conventional processes for lactic acid production (Lipinsky & Sinclair, 1986). Lactate esters derived from biolactic acid are being considered as alternative benign solvents (Watkins, 2002).

Lactic acid can be produced by fermentation of biomass. In conventional processes, lactic acid is recovered from the fermentation broth by the precipitation of calcium lactate with calcium hydroxide. In this, the separation and final

purification stages account for up to 50% of the production costs (Chaudhuri & Pyle, 1992; Eyal & Bressler, 1993). Thus, this method of recovery is expensive and unfriendly to the environment as it consumes lime and sulfuric acid and also produces a large quantity of calcium sulfate sludge as solid waste (Shreve & Brink, 1977). Allowing accumulation of lactic acid products in the fermentation broth inhibits further product formation. The effects of end product inhibition can be reduced by in situ removal of lactic acid from the fermentation broth by several methods.

Reactive extraction with extractant giving a higher distribution coefficient has been proposed as a promising technique for the recovery of carboxylic and hydroxycarboxylic acids (Wardell & King, 1978; Wennersten, 1983). Reactive liquid–liquid extraction has the advantage that lactic acid can be removed easily from the fermentation broth, preventing the lowering of pH. Further, lactic acid can be re-extracted and the extractant can be recycled to the fermentation process. Tertiary amines are found to be effective extractants (Ratchford, Harris, Fisher, & Willits, 1951; King, 1983). Alamine 336 (mixture of C₈, C₉ and C₁₀ tertiary amines)

* Corresponding author. Tel.: +91-22-414-5616;

fax: +91-22-414-5614.

E-mail address: vgp@udct.ernet.in (V.G. Pangarkar).

yields a good combination of high K_D , very low solubility in water and good regenerability.

Enzymatic production of lactic acid from glucose by fermentation represents one of the few commercially established enzymatic processes. Optimum utilization of the biocatalysts requires not only immobilization of the enzyme for multiple uses but also a rational design of the reactor to operate in conditions of maximum stability. pH has been known to be the most important parameter related to the stability of the enzyme. When glucose is converted into lactic acid the pH falls. As a result the rate of enzymatic reaction decreases and if the pH drops further, deactivation of enzyme is accelerated. Therefore, it is essential to control the pH by the addition of an alkali so that the rate of reaction is maintained at its maximum. The enzymatic reaction suffers not only substrate inhibition but also competitive inhibition by lactic acid. Hence, with the 'in situ extraction' of the lactic acid within the fermentation vessel, the enzymatic reaction rate is increased.

The design of an amine extraction process requires (i) equilibrium and (ii) kinetic data for the acid–amine (solvent) system used. Considerable information on the equilibrium of several acid–amine (solvent) systems is available in the literature. Some information pertaining to kinetics is available in the literature (Wasewar, Heesink, Versteeg, & Pangarkar, 2002a,b) on MIBK and decanol as diluents. Octanol can also be used as a diluent and it is likely that it may yield K_D and rate constant higher than the previously reported diluents (Wasewar et al., 2002a, b). This study reports the liquid–liquid equilibrium data and kinetics for the extraction of lactic acid by Alamine 336 (a tertiary amine, with aliphatic chains of 8–10 carbon groups) using octanol as a diluent. These data have been used for modeling the process of enzymatic fermentation accompanied by reactive extraction. The approach for intensification of hydrolysis of Penicillin G reported by Gaidhani, Wasewar, & Pangarkar (2002a), Gaidhani, Tolani, Pangarkar, & Pangarkar (2002b) is used here for the intensification of fermentation and recovery of lactic acid from the fermentation broth. A mathematical model has been developed for this slurry phase reactor with glucose in the continuous aqueous phase, the amine dissolved in a diluent in the dispersed organic phase and the immobilized enzyme as the solid catalyst. The model has been solved for batch and semi-batch modes for the study of performance of reactor for in situ recovery of lactic acid from fermentation broth by reactive extraction. Effects of various parameters affecting the conversion of glucose have been discussed.

2. Materials and methods

2.1. Materials

All the chemicals used (lactic acid, octanol, sodium hydroxide) were of reagent grade and used without

pretreatment. Lactic acid, NaOH and octanol were supplied by s. d. fine chemicals Ltd. Mumbai, India. All solutions of lactic acid were prepared by dissolving lactic acid of analytical purity with distilled water. Low concentration range (0.005 – 1.5 kmol m^{-3}) was used because in the practical case of acid recovery from fermentation broths, the acid concentrations are not expected to be high.

The reactive component was Alamine-336 (straight chain tertiary amine containing C_8 – C_{10} alkyl groups (Henkel Corp., USA), with octanol as a diluent. Alamine was used as supplied.

2.2. Methods

Analytical techniques for measuring lactic acid concentration, method of equilibria and kinetic studies have been presented previously (Wasewar et al., 2002b).

The reproducibility was checked by carrying out duplicate experiments in some cases. The results were found to be reproducible within $\pm 3\%$.

3. Results and discussion

3.1. Extraction equilibria

The equilibrium distribution of lactic acid in octanol and octanol with various concentrations of Alamine-336 was measured at 30°C and compared with other diluents MIBK (Wasewar et al., 2002a) and decanol (Wasewar et al., 2002b). Distribution coefficients obtained by a statistical analysis of the equilibrium data of lactic acid for various Alamine-336 concentrations in octanol and other diluents are given in Table 1. The equilibrium complexation constant of (1:1) lactic acid–Alamine complex was estimated and is given in Table 2 with other diluents.

Tables 1 and 2 indicate that the values of distribution coefficient and equilibrium complexation constant for octanol are the highest. Thus, octanol is a better solvent than the other two on equilibrium consideration. The differences among distribution coefficient and equilibrium values for the same acid in different diluents indicate that solvation of

Table 1
Distribution coefficient for lactic acid extraction with various concentrations of Alamine-336 in different diluents

% Alamine-336	Distribution coefficient, K_D		
	MIBK ^a	Decanol ^b	Octanol
0	0.31	0.13	0.29
10	—	—	15.35
20	0.72	12.57	19.69
30	2.68	16.44	25.95
40	4.24	23.37	—

^aWasewar et al. (2002a).

^bWasewar et al. (2002b).

Table 2
Equilibrium complexation constant of lactic acid–Alamine-336 complex in various diluents

Diluent	Complexation constant
MIBK ^a	10.15 m ³ kmol ⁻¹
Decanol ^b	45.21 m ³ kmol ⁻¹
Octanol	73.55 m ³ kmol ⁻¹

^aWasewar et al. (2002a).

^bWasewar et al. (2002b).

the complex by the diluent is a critical factor in the extraction of the acid. The interactions between the complex and solvent can, somewhat arbitrarily, be divided into ‘general solvation’ and ‘specific interaction’ of the diluent with the complex. Details of these interactions are given by Tamada and King (1990).

3.2. Kinetics

Doraiswamy and Sharma (1984) have given an exhaustive discussion of the theory of extraction accompanied by a chemical reaction. Four regimes of extraction accompanied by reaction (very slow, slow, fast and instantaneous) have been identified depending upon the physico-chemical and hydrodynamic parameters. Doraiswamy and Sharma (1984) have given the guidelines for discerning the mechanism. The expression for Regime 3, extraction accompanied by a fast general order chemical reaction occurring in the diffusion film is

$$R_A = [A^*] \sqrt{\frac{2}{m+1}} D_A k_{mn} [A^*]^{m-1} [B]_{\text{org}}^n. \quad (1)$$

3.2.1. Physical mass transfer coefficient

The value of physical mass transfer coefficient k_L is required for confirming the regime of extraction. This was obtained by conducting a physical extraction (diluent only) of lactic acid from water as reported previously (Wasewar et al., 2002b). The regression relation between mass transfer coefficient and speed of agitation obtained by a statistical analysis of data is

$$k_L = 2 \times 10^{-6} N^{0.4}. \quad (2)$$

3.2.2. Reaction Regime

The reaction between lactic acid and Alamine-336 is reversible particularly under conditions of high loading of lactic acid complex with the amine in the organic phase. To avoid problems due to this reversibility only initial rates were considered for evaluation of the kinetics.

Effect of speed of agitation. The speed of agitation was varied from 0.6–1.4 rev s⁻¹. In this range the liquid–liquid interface was flat and the interfacial area for extraction was equal to the geometric area. Fig. 1 indicates that there was no effect of speed of agitation on the specific rate of

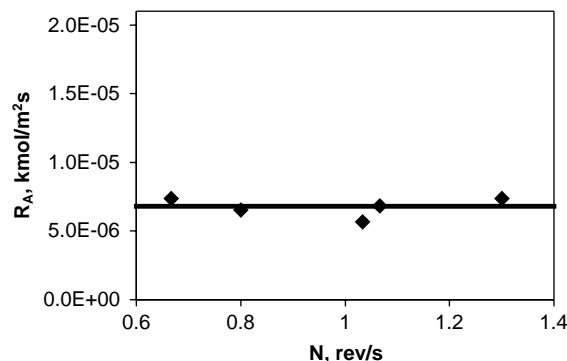


Fig. 1. Effect of speed of agitation on specific rate of extraction for the reactive extraction of lactic acid with Alamine-336 in octanol. Initial lactic acid concentration=0.38 kmol m⁻³, Alamine concentration in organic phase=0.41 kmol m⁻³, Temp.=30°C.

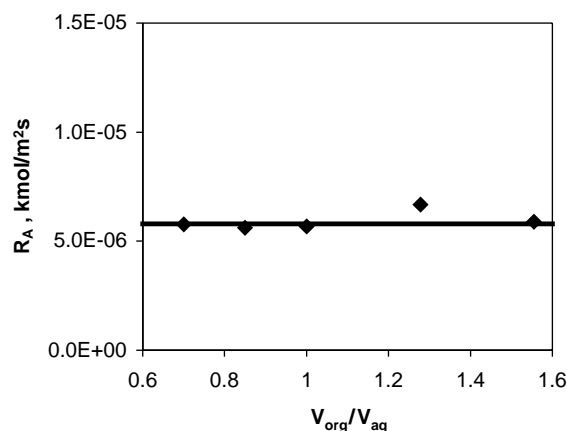


Fig. 2. Effect of phase ratio on specific rate of extraction for the reactive extraction of lactic acid with Alamine-336 in octanol. Initial lactic acid concentration=0.38 kmol m⁻³, Alamine concentration in organic phase=0.41 kmol m⁻³, Speed of agitation=1.03 rev s⁻¹, Temp.=30°C.

extraction, R_A (kmol m⁻² s⁻¹). This situation is possible if either Regime 1 or Regime 3 described by Doraiswamy and Sharma (1984) is valid.

Effect of phase volume ratio. To differentiate between Regimes 1 and 3, the effect of organic phase volume on the specific rate of extraction was studied. Fig. 2 shows the plot of R_A vs. phase volume ratio (volume of organic phase/volume of aqueous phase) at a constant speed of agitation. Evidently, there is no effect of phase volume.

From the above experimental results, it can be concluded that the reaction between lactic acid and Alamine-336 in octanol in a stirred cell falls in Regime 3, extraction accompanied by a fast chemical reaction occurring in the diffusion film.

3.2.3. Order of reaction

Order with respect to Alamine-336. Fig. 3 shows a plot of the specific rate of extraction of lactic acid against initial Alamine-336 concentration in the organic phase. Evidently

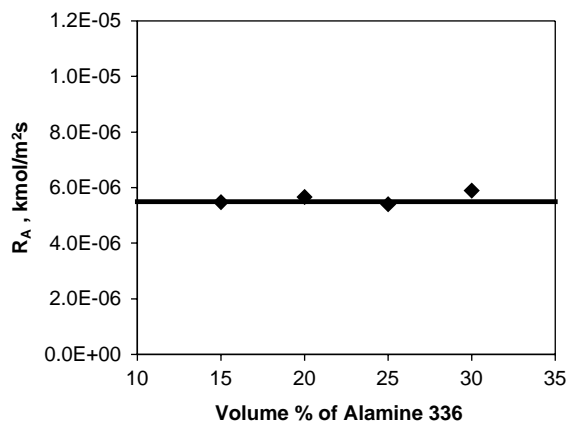


Fig. 3. Effect of initial Alamine-336 concentration on specific rate of extraction for the reactive extraction of lactic acid with Alamine-336 in octanol. Initial lactic acid concentration = 0.38 kmol m^{-3} , Speed of agitation = 1.03 rev s^{-1} , Temp. = 30°C .

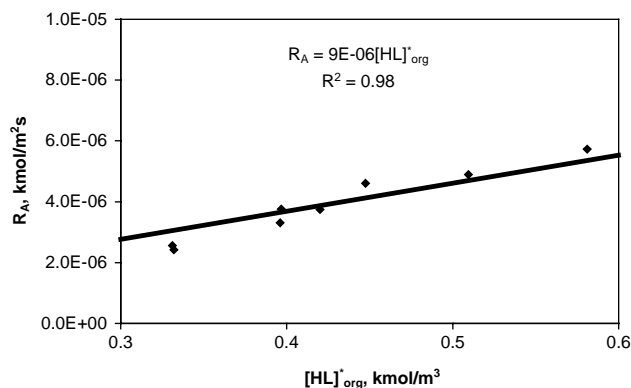


Fig. 4. Effect of initial lactic acid concentration on specific rate of extraction for the reactive extraction of lactic acid with Alamine-336 in octanol. Alamine concentration in organic phase = 0.41 kmol m^{-3} , Speed of agitation = 1.03 rev s^{-1} , Temp. = 30°C .

there is no effect of Alamine-336 concentration on the rate of extraction indicating that the reaction is zero order in Alamine-336 ($n = 0$ in Eq. (1)).

Order with respect to lactic acid. Fig. 4 shows the effect of organic phase lactic acid concentration on specific rate of extraction, R_A . A regression analysis of the data yielded $m = 1$ (as per Eq. (1)). Thus, the reaction is first order with respect to lactic acid.

3.2.4. Rate constant

For $m = 1$ and $n = 0$, Eq. (1), the rate expression for the initial part of the extraction is reduced to

$$R_A = [HL]_{\text{org}}^* \sqrt{D_A k_1}.$$

The data were fitted to the above equation to obtain the value of the first-order rate constant (Fig. 4), k_1 as 24 s^{-1} . The value of D_A was estimated using Wilke and Chang, (1955) equation as $1.25 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$.

Table 3

Rate constants of lactic acid–Alamine-336 reaction in various diluents

Diluent	Rate constant (s^{-1})
MIBK ^a	1.4
Decanol ^b	0.2
Octanol	24

^aWasewar et al. (2002a).

^bWasewar et al. (2002b).

To confirm the validity Regime 3, the value of the parameter $\sqrt{(D_A k_1)}/k_L$ should be greater than 3 (Doraiswamy and Sharma, 1984).

The range of k_L is 1.5×10^{-6} to $2 \times 10^{-6} \text{ m s}^{-1}$ and value of $\sqrt{D_A k_1}$ from Fig. 4 is $9 \times 10^{-6} \text{ m s}^{-1}$. Hence the range of value of $\sqrt{(D_A k_1)}/k_L$ is 4.5–6. Thus the condition for the validity of Regime 3 is satisfied. Therefore, the above-mentioned results reflect the intrinsic kinetics of the extraction process.

3.2.5. Comparison with other diluents

Rate constant of lactic acid–Alamine-336 reaction in octanol and in other diluents MIBK (Wasewar et al., 2002a) and decanol (Wasewar et al., 2002b) are given in Table 3. Table 3 indicates that based on kinetic consideration octanol is a better solvent than the other two solvents.

4. Modeling

4.1. Model development

There are essentially two different reactions occurring at two different interfaces :

At the solid (cat.)–liquid (aqueous) interface:

Glucose is converted to lactic acid. It is assumed that this reaction is a kinetically controlled reaction with substantial intra-particle diffusional resistance depending on the enzyme and support used. In the earlier work on hydrolysis of Penicillin G, a similar situation was considered (Gaidhani et al., 2002b).

At the liquid-liquid interface:

Lactic acid is generated in Step 1 complexes with the alamine in the dispersed phase. This will be shown to be kinetic controlled at a later stage.

The kinetic expression for the lactic acid production from glucose by *Rhizopus oryzae* has been given by Sun, Li and Bai (1999) as

$$R = \frac{R_{\text{max}}[S]}{K_S + S + \frac{[S]^2}{K_M}} \left(1 - \frac{[P]}{K_P} \right), \quad (3)$$

where R is the specific rate of formation of lactic acid in $\text{kg kg}^{-1} \text{ h}^{-1}$, R_{max} the maximum rate of lactic acid formation ($4.83 \text{ kg kg}^{-1} \text{ h}^{-1}$), K_M the Michaelis–Menten

constant (66.9 kg m^{-3}), K_S the substrate saturation constant (8.79 kg m^{-3}), K_P the product inhibition constant (208 kg m^{-3}), Y_{PS} the lactic acid yield based on glucose consumption (0.6).

Effect of pH within the particle, i.e. the effect on the enzyme and on the carrier matrix have not been considered individually. Thus the pH effects have been ‘lumped’ with the kinetic terms of the model, resulting in pH-dependent kinetic parameters. In the immobilized catalyst, intra-particle diffusional resistance offered by the enzyme and support substantially affects the overall rate. Traditionally, the importance of diffusional resistance in such situations is quantified using the Thiele modulus. Bischoff (1965) has developed the procedure for extending the Thiele modulus to general kinetics. However, for the rate equation (3), the resultant expression arrived at following such a treatment is very complex and not amenable to analytical solution. Consequently, a simplified form of the Thiele modulus for the Michaelis–Menten kinetics using the method given by Bischoff (1965) can be defined as

$$\phi = \frac{r}{3} \left(\frac{R_1 \max}{2K_M D_e} \right)^{0.5} \frac{[S]}{K_M + [S]} \times \left\{ [S] - K_M \ln \left(1 + \frac{[S]}{K_M} \right) \right\}^{-0.5}, \quad (4)$$

where $r = 3 \text{ mm}$ and $D_e = 7 \times 10^{-14} \text{ m}^2 \text{ s}^{-1}$ was used for this study (Sun, Li & Bai, 1999).

The effectiveness factor η is given by

$$\eta = \frac{\tanh \phi}{\phi}. \quad (5)$$

Activity of the enzyme depends on the pH. The effect of pH on the specific growth rate has been given by many workers (Yeh, Bajpai, & Iannotti, 1991, Roy, Goulet, & Leduy, 1987, Payot, Chemaly, & Fick, 1999, Fu & Mathews, 1999). An activity factor (a_f) is introduced to consider the effect of actual pH of the system on the activity of the enzyme. Activity factor is defined as the ratio of specific rate of reaction to the maximum rate of reaction (rate at optimum pH).

$$a_f = \frac{\text{Specific rate of reaction}}{\text{Specific rate of reaction at optimum pH}}. \quad (6)$$

Activity factor–pH correlation has been developed by regression technique using the experimental data of Roy et al. (1987). The correlation is

$$a_f = 0.033 \text{ pH}^{1.97}. \quad (7)$$

It is evident that a_f is a strong function of pH. The maximum rate of reaction (R_{\max}) considered is for 100% activity of the enzyme, i.e. at $\text{pH}=5.56$ (Roy et al., 1987).

Hence, the rates of enzymatic reaction using immobilized biocatalyst are as follows:

Rate of formation of lactic acid,

$$R_P = \frac{d[P]}{dt} = R\eta[\text{EnZ}]a_f. \quad (8)$$

Rate of consumption of substrate (glucose),

$$R_S = \frac{d[S]}{dt} = \frac{1}{Y_{PS}} R\eta[\text{EnZ}]a_f. \quad (9)$$

The above is an unsteady-state model describing diffusion and reaction within a spherical pellet of immobilized biocatalyst. When such pellets are used in a batch reactor, they see a changing environment. Hence, the particle level equations are to be coupled with the material balance equations set up in the bulk fluid.

The reaction regime of lactic acid–Alamine-336 in a stirred cell is Regime 3. The actual regime of extraction in an agitated reactor may be different from a stirred cell, since the former offers much higher value of ‘ $k_L a$ ’ and ‘ a ’ than in a stirred cell. If values of ‘ k_L ’ and ‘ a ’ in the lower range (Doraiswamy & Sharma, 1984) are considered then in a mechanically agitated contactor,

$$\sqrt{M} = \frac{\sqrt{k_1 \times D_A}}{k_L} \approx 0.8 \text{ and } k_L a = 10 > \varepsilon_D k_1 = 1.5.$$

Thus, it can be assumed that the reaction falls in Regime 1, kinetic controlled slow reaction (Doraiswamy & Sharma, 1984).

Hence, the rate expression for extraction is

$$R_A = V\varepsilon_D k_1 ([P^*] - [P]_0). \quad (10)$$

Thus taking the mass balance on lactic acid,

Rate of accumulation in the continuous phase

= Rate of generation in the continuous phase

– Rate of extraction in the dispersed phase

$$V\varepsilon_L = \frac{d[P]}{dt} = \varepsilon_L V R_P - V\varepsilon_D k_1 ([P^*] - [P]_0). \quad (11)$$

Conversion of glucose X is defined as

$$X = \frac{[S]_0 - [S]}{[S]_0} \times 100. \quad (12)$$

The productivity of the enzymatic conversion of glucose to lactic acid is an important parameter which directly reflects the extent of intensification of the enzymatic reaction by the simultaneous reactive extraction. The productivity of lactic acid is defined as the amount of lactic acid produced per unit reactor volume per unit time ($\text{kg m}^{-3} \text{ h}^{-1}$).

A program in Fortran77 was written to solve the differential equations simultaneously using the fourth-order

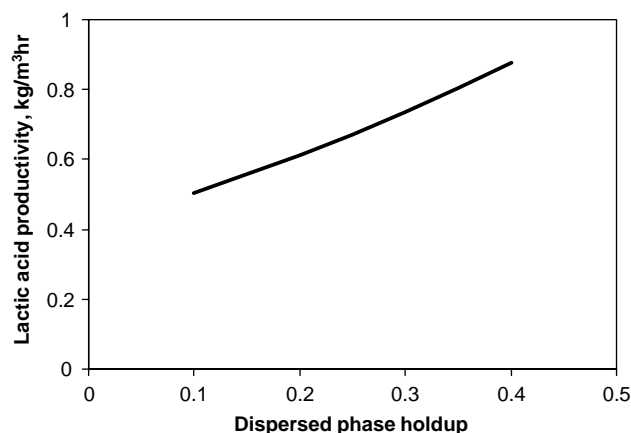


Fig. 5. Effect of organic phase holdup on productivity in batch mode. $S_0 = 30 \text{ kg m}^{-3}$, $[\text{EnZ}] = 2 \text{ kg m}^{-3}$, $\varepsilon_S = 0.1$, initial Alamine=15%.

Runge Kutta method. The methodology for solving the model equations has been reported previously (Gaidhani et al., 2002b).

4.2. Reactor performance

4.2.1. Batch mode

In this mode, the entire amount of glucose, Alamine-336 with diluent and enzyme catalyst are taken at the start of the batch, which is then processed. The reaction occurs at the enzyme surface and lactic acid is formed. Lactic acid diffuses to the aqueous phase and is then extracted by the organic phase. There is a finite amount of Alamine-336 in the organic phase and as the extraction continues the equilibrium concentration of lactic acid in the organic phase increases. This results in a decrease in the driving force for the extraction of lactic acid, which consequently slows down. This causes a buildup of lactic acid concentration in the aqueous phase and thereby decreases its pH. This lowering of pH has an adverse effect on the rate of the enzymatic reaction which slows down. Similar results are reported by Gaidhani et al. (2002b) for the hydrolysis of Penicillin G.

Effect of organic phase hold-up, ε_D . For unit volume of the reactor, $\varepsilon_D + \varepsilon_L + \varepsilon_S = 1$. Thus, it is not possible to vary ε_D without affecting either ε_L or ε_S . The calculation for this case were performed by varying ε_D at constant ε_S but variable ε_L . Fig. 5 shows the effect of dispersed phase holdup (ε_D) on the productivity of lactic acid. It is evident that increasing ε_D increases the conversion. The effect of ε_D increases as the reaction proceeds. This is due to the lower equilibrium concentration of lactic acid in the organic phase at higher ε_D , which keeps the driving force for lactic acid extraction at a higher level. At very low conversion, where the lactic acid–Alamine-336 reaction is essentially irreversible, the effect of ε_D is minimal. It is evident from Fig. 5 that even at a high $\varepsilon_D = 0.4$, the productivity of lactic acid is only $0.87 \text{ kg m}^{-3} \text{ h}^{-1}$. Further increase in ε_D can increase the rate of extraction but the productivity of the

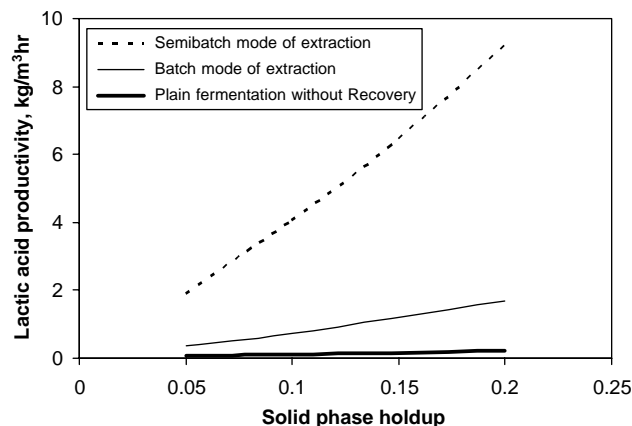


Fig. 6. Effect of solid phase holdup on productivity of lactic acid. Batch mode : $S_0 = 30 \text{ kg m}^{-3}$, $[\text{EnZ}] = 2 \text{ kg m}^{-3}$, $\varepsilon_D = 0.3$, initial Alamine=15%; semibatch mode: $S_0 = 30 \text{ kg m}^{-3}$, $[\text{EnZ}] = 2 \text{ kg m}^{-3}$, initial Alamine=15%.

reactor will suffer since the amount of glucose taken in the batch is related to ε_L ($\varepsilon_L = 1 - \varepsilon_D - \varepsilon_S$). In this scheme, ε_D increases at the expense of ε_L and with decreasing ε_L the quantity of glucose employed decreases. Thus, it is not advisable to have a higher ε_D .

Effect of solid (catalyst) loading. Fig. 6 shows the effect of ε_S on the conversion of Glucose. Industrially an ε_S of 0.08–0.1 is considered practical. Evidently, there is a sharp increase in the reaction rate as ε_S increases. This is due to increased enzyme concentration. However, the productivity is only $0.74 \text{ kg m}^{-3} \text{ h}^{-1}$ at a solid loading of 0.1, which again is due to the batch nature of the extraction of lactic acid and reversibility of the reaction with Alamine-336, as explained earlier.

Effect of initial Glucose concentration. There are two effects of increased glucose concentration. With increasing glucose concentration (a) the substrate inhibition assumes greater importance and (b) the lactic acid formed increases and at high loadings of lactic acid in the organic phase the reversible nature of the extraction becomes dominant. The overall effect of these two factors is to lower the final conversion of Glucose. Thus, at 30 kg m^{-3} of glucose in the feed the productivity is $0.74 \text{ kg m}^{-3} \text{ h}^{-1}$ whereas at 10 kg m^{-3} of glucose the same is only $0.47 \text{ kg m}^{-3} \text{ h}^{-1}$ (Fig. 7).

Effect of concentration of Alamine-336 in the organic phase. The calculations were performed at four different Alamine-336 concentrations. The results are shown in Fig. 8. Evidently, in the range of concentration studied there is no effect of Alamine-336 concentration. The concentration generally accepted is 15–25 wt% of Alamine-336. At higher concentration the organic phase has a higher viscosity, which is detrimental as it lowers the diffusivity of the solute.

4.2.2. Semi-batch mode

In this mode, the glucose solution and the catalyst are taken in batch and the extracting organic phase is

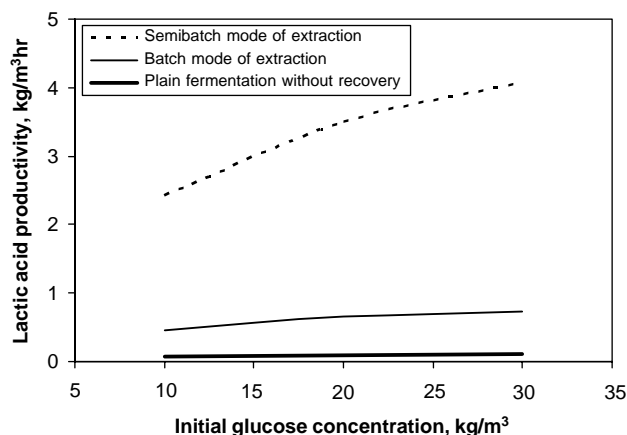


Fig. 7. Effect of glucose concentration on productivity of lactic acid. Batch mode: $[\text{EnZ}] = 2 \text{ kg m}^{-3}$, $\varepsilon_S = 0.1$, $\varepsilon_D = 0.3$, initial Alamine=15%; semibatch mode: $[\text{EnZ}] = 2 \text{ kg m}^{-3}$, $\varepsilon_S = 0.1$, initial Alamine=15%.

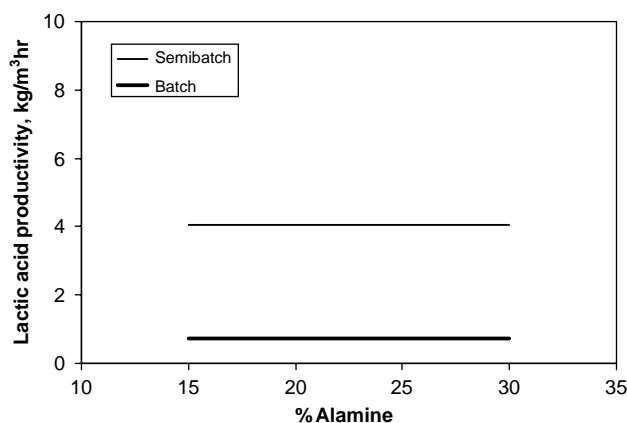


Fig. 8. Effect of Alamine concentration on productivity of lactic acid. Batch mode: $S_0 = 30 \text{ kg m}^{-3}$, $[\text{EnZ}] = 2 \text{ kg m}^{-3}$, $\varepsilon_S = 0.1$, $\varepsilon_D = 0.3$.

continuously fed and withdrawn. The loaded organic phase is taken out and regenerated in a separate vessel. The organic phase extracts the lactic acid formed as in the previous case. However, since the organic phase is flowing in and out continuously there is a limited loading and, hence there is lower equilibrium concentration in this case. Similar approach was used by Gaidhani et al. (2002b) for the hydrolysis of Penicillin G. The model required information on the holdup of the dispersed phase, ε_D , as a function of power input/volume (P/V), the dispersed phase velocity, V_D , and the solid loading. The following correlation for ε_D given by Gaidhani et al. (2002b) was used:

$$\varepsilon_D = 1.4(P/V)^{0.44}(V_D)^{0.78}(\varepsilon_S)^{0.13}, \quad (13)$$

where (P/V) is in kW m^{-3} , V_D is in m s^{-1} and ε_S is the solid catalyst holdup (volume fraction).

Effect of pH. In the semi-batch mode, the pH of the aqueous phase is the only variable. Both the continuous and dispersed phases are assumed to be completely mixed. The

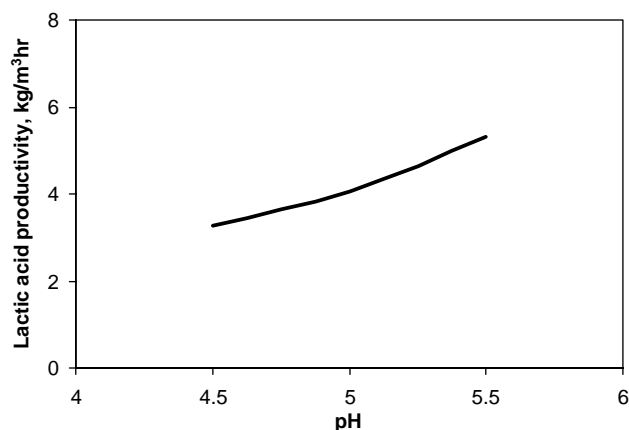


Fig. 9. Effect of pH on productivity of lactic acid in semi batch mode. $S_0 = 30 \text{ kg m}^{-3}$, $[\text{EnZ}] = 2 \text{ kg m}^{-3}$, $\varepsilon_S = 0.1$, initial Alamine=15%. $S_0 = 30 \text{ kg m}^{-3}$, $[\text{EnZ}] = 2 \text{ kg m}^{-3}$, initial Alamine=15%.

calculations were performed by assuming a pH of operation and required dispersed phase velocity, since the pH and V_D effects are closely interlinked and cannot be separated. This mode of operation is similar to that used by Yabannavar and Wang (1991) for extractive fermentation of lactic acid. However, in the present case, similar to Gaidhani et al. (2002b), reaction and extraction occur in the same reactor.

Fig. 9 shows the effect of pH on lactic acid productivity. It is evident that a higher pH has beneficial effect. However, with increasing pH there is an increase in V_D due to the fact that higher pH implies lower lactic acid concentration in the aqueous phase. This lowers the driving force for extraction; to combat it there is a need to increase ε_D through increase in V_D . At higher V_D , the concentration of the lactic acid–amine complex in the organic phase is lower. This has a direct effect on the processing cost for recovery of lactic acid from the organic phase (Yabannavar & Wang, 1991).

Effect of glucose concentration. As mentioned earlier the reaction is also inhibited by the substrate, glucose. Thus, higher feed concentration of glucose increases the time for a given level of conversion. The results are shown in Fig. 7. Comparison of semi-batch, batch and plain fermentation without recovery operation clearly indicates the superiority of semi-batch operation. For instance at 10 kg m^{-3} glucose in the feed, productivity of lactic acid is $2.42 \text{ kg m}^{-3} \text{ h}^{-1}$ in semi-batch, $0.47 \text{ kg m}^{-3} \text{ h}^{-1}$ in batch and $0.1 \text{ kg m}^{-3} \text{ h}^{-1}$ for plain fermentation without recovery operation.

Effect of enzyme loading. The superiority of the semi-batch mode is evident in this case also. Fig. 6 shows the effect of solid loading on lactic acid productivity. For $\varepsilon_S = 0.1$, productivity of lactic acid is $0.74 \text{ kg m}^{-3} \text{ h}^{-1}$ in batch mode and $0.1 \text{ kg m}^{-3} \text{ h}^{-1}$ in without recovery operation. For the same conditions the semi-batch mode yields $4 \text{ kg m}^{-3} \text{ h}^{-1}$.

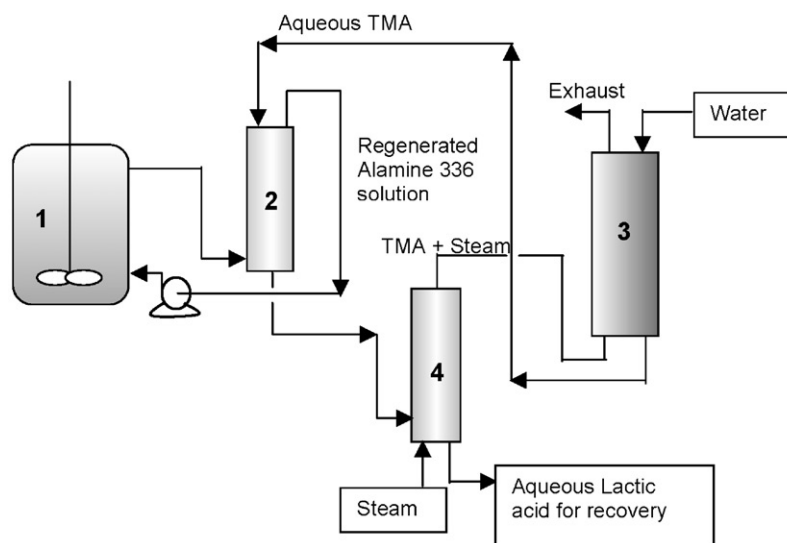


Fig. 10. Process flowsheet for the production of lactic acid by enzymatic fermentation accompanied by reactive extraction and recovery of lactic acid. 1: fermenter, 2: regenerator for Alamine 336, 3: absorber for TMA, 4: stripper for TMA, TMA: trimethylamine.

4.3. Process flow sheet

Fig. 10 shows the process flow sheet envisaged from which it is clear that a completely eco-friendly, sustainable and efficient process for the production of lactic acid can be designed. Work on back extraction of lactic acid by TMA (trimethylamine) is in progress and will be reported in a separate communication.

5. Conclusion

Equilibrium and kinetic data for extraction of lactic acid in Alamine-336 using octanol as a diluent are reported. Comparison of these data with MIBK and decanol as diluent suggests that octanol is a better diluent than MIBK and decanol on both equilibrium and kinetic considerations.

A method for more efficient conversion of glucose to lactic acid has been suggested. This method uses Alamine-336 in octanol to extract lactic acid. A model has been developed to predict the effects of various operating parameters on the conversion of glucose and reactor performance. The results clearly indicate the superiority of the semi-batch mode of operation with respect to extraction. Lactic acid extracted in the organic phase can be extracted back with a stronger volatile amine like TMA in aqueous phase. The TMA can be stripped and recovered by absorption in water, and recycled to obtain a closed-loop system. The process suggested is a sustainable process as it does not consume extra reagent and also does not produce a large waste stream as in the conventional process.

Notation

a	interfacial area of the dispersed phase per unit volume of the three-phase system, $\text{m}^2 \text{m}^{-3}$
a_f	activity factor, (dimensionless)
$[A^*]$	equilibrium concentration in organic phase, kmol m^{-3}
$[B]_0$	Alamine 336 concentration in the organic phase, kmol m^{-3}
D_A	molecular diffusivity of lactic acid in octanol, $\text{m}^2 \text{s}^{-1}$
D_e	effective diffusivity of glucose, $\text{m}^2 \text{s}^{-1}$
$[\text{EnZ}]$	concentration of enzyme, kg m^{-3}
$[\text{HL}]$	lactic acid concentration, kmol m^{-3}
$[\text{HL}]_{\text{org}}^*$	equilibrium concentration of lactic acid in organic phase, kmol m^{-3}
k_1	first-order reaction rate constant for the complexation reaction between lactic acid and Alamine 336 in octanol, s^{-1}
k_L	mass transfer coefficient, m s^{-1}
k_{mn}	Rate constant for a reaction that is m th order in species A and n th order in species B, $(\text{m}^3 \text{kmol}^{-1})^{n+m-1}$
K_D	distribution coefficient
K_M	Michaelis–Menten constant in rate Eq. (3), kg m^{-3}
K_P	product inhibition constant, kg m^{-3}
K_S	substrate saturation constant, kg m^{-3}
N	speed of agitation, rev s^{-1}
P	power input, kW
$[P^*]$	saturation concentration of lactic acid in the diluent, kg m^{-3}
$[P]$	lactic acid concentration, kg m^{-3}

$[P]_0$	equilibrium concentration of lactic acid in the organic phase, kg m^{-3}
r	radius of the particle, m
R	specific rate of formation of lactic acid, $\text{kg kg}^{-1} \text{h}^{-1}$
R_A	specific rate of extraction of lactic acid, $\text{kmol m}^{-2} \text{s}^{-1}$
R_{\max}	maximum rate of formation of lactic acid, $\text{kg kg}^{-1} \text{h}^{-1}$
R_P	rate of formation of lactic acid, $\text{kg m}^{-3} \text{h}^{-1}$
R_S	rate of consumption of substrate (glucose), $\text{kg m}^{-3} \text{h}^{-1}$
$[S]$	substrate (glucose) concentration, kg m^{-3}
$[S]_0$	initial substrate (glucose) concentration, kg m^{-3}
t	time of extraction, s
V	volume of reactor, m^3
V_{aq}	volume of aqueous phase, m^3
V_{org}	volume of organic phase, m^3
V_D	superficial velocity of dispersed phase, m s^{-1}
X	conversion of glucose in %
Y_{PS}	lactic acid yield based on glucose consumption

Greek letters

$\varepsilon_D, \varepsilon_L, \varepsilon_s$	dispersed, continuous and solid phase volume fractions, respectively
η	effectiveness factor (dimensionless)
ϕ	Thiele modulus (dimensionless)

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